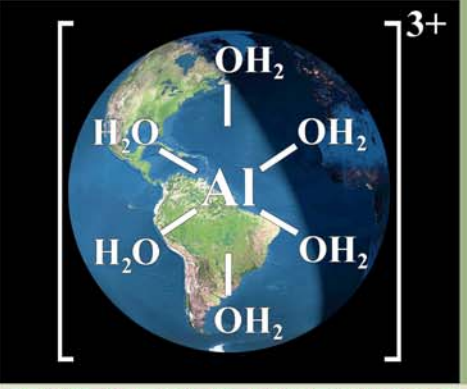

Birchall Centre for Inorganic Chemistry and Materials Science,
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and
Czech University of Life Sciences in Prague

Eighth Keele Meeting on Aluminium

THE KEELE MEETINGS ON ALUMINIUM 1995, 1997, 1999, 2001, 2003, 2005, 2007

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

KEELE UNIVERSITY

The Natural History of Aluminium:

From Non-Selection to Natural Selection

Book of Abstracts

Saturday 21st to Wednesday 25th February 2009

Castle Hotel, Třešť, Czech Republic

http://www.keele.ac.uk/depts/ch/groups/aluminium/index.html

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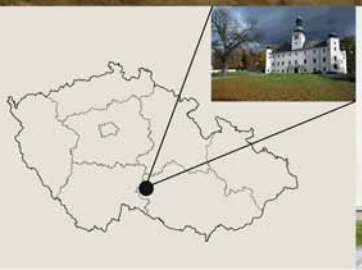

Department of Soil Science and Soil Protection
CZECH UNIVERSITY OF LIFE SCIENCES IN PRAGUE

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Editors: Christopher Exley
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Contents

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SESSION 1. ALUMINIUM CHEMISTRY

Platform presentations

Platform 1

Interfacial behaviour of sapphire basal planes

Johannes Lützenkirchen[a], Thomas Rabung[a], Dieter Schild[a], Andre Filby[a], Marcus Plaschke[a], Horst Geckeis[a], Ralf Zimmermann[b], David Küttner[b], Carsten Werner[b,c], Tajana Preocanin[d]

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We discuss the interfacial behaviour of α-Al_2O_3 basal planes in electrolyte solutions. The perfect, O-terminated, hydroxylated surface should consist of only doubly-coordinated hydroxyls (DCHs), i.e. a well-suited surface for the understanding of interfacial charging processes. Based on the popular MUSIC model these DCHs are not proton active over a wide pH range. The point of zero charge is expected at pH 6 and the surface potential should exhibit a plateau between pH 4.5 and 7.5. A wide range of data related to the interfacial charging of this plane have been obtained with various techniques including non-linear optics, atomic force microscopy, streaming potential and current measurements, colloid adsorption and contact angle titration. Most of these data show sharp points of zero charge below pH 5. In order to explain why the charging of these "ideal" model systems is very different from those of corresponding particles we discuss the available data with respect to water ion adsorption.

Platform 2

Glyphosate complexation to aluminium(III). Equilibrium and structural study in solution by potentiometry, multinuclear NMR, ESI-MS and DFT calculation

Imre Tóth[a], Zoltán Takács,[a,b], Mihály Purgel,[a] István Bányai[a], Imre Pápai[c], Ingegärd Andersson[b], Staffan Sjöberg[b]

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Glyphosate (N-phosponomethyl glycine, PMG) has been used in vast amounts in the agriculture during the last few decades. Decomposition/transport of this non-selective herbicide in nature could strongly be dependent on chemical speciation, i.e. complexation to metal ions in solution and/or surfaces of soil components. As part of a broader project we have studied the aqueous Al(III)-PMG system using different experimental tools and theoretical calculations (density function theory, DFT).

Al(III) forms very stable complexes with PMG (L^{3-}). The combined potentiometric–NMR (^{27}Al and ^{31}P) measurements have shown that among some mono-nuclear complexes (e.g. AlH_2L^{2+}, $AlHL_2^{2-}$) di-nuclear ($Al_2HL_2^{+}$) and tri-nuclear ($Al_3H_5L_4^{2+}$) complexes are likely formed in contrast to previous investigations. ESI – QqTOF spectra confirm the formation of these poly-nuclear complexes. ^{31}P and ^{1}H NMR spectra indicate the presence of several structural isomers of the species. DFT calculations resulted in reasonable structures of the different complexes formed.

Platform 3

Supramolecular assemblies of Al^{3+} complexes. Encapsulation and complexation studies of B-cyclodextrin with cholecalciferol (vitamin D3) and phenothiazine

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Supramolecular assembly compounds can have myriads of uses in the daily life. When these compounds encompasses B-cyclodextrins as hosts the already in the literature studied biocompatibilities are suffice to put these compounds into the realm of safe substances. These includes new drugs encapsulated as guests in macromolecules that can be delivered by decomplexation at specific environmental conditions. On the other hand, although the costs are not too promising, these molecules can also play an important role in the remediation of either water or soil with the degradation products of agrotoxics and with aluminum contamination. This metal ion is spread in the 3+ form in the nature by the industrialization processes specially of foods, and also in the municipal treated waters by the use of aluminum sulphate as flocculant. The encapsulation of either vitamin D3 for the purpose of obtaining a specifically delivered drug and either phenothiazine, a degradation product of some agrotoxics, were studied in B-cyclodextrins in the absence and in the presence of Al^{3+}. The analytical methods employed were two. Potentiometric titrations in order to obtain the stability constants of the equilibria with the encapsulated products in the presence of Al^{3+} and the values of pH where these complexes were formed and ended(speciation according to different pHs). ^{13}C NMR, to monitor the formation of these assemblies and the maintenance of the supramolecular structure in the solid state. for practical purposes. The ^{13}C NMR results showed that both supramolecular structures were successfully obtained and the potentiometric studies could provided the data to calculate the binding constants of the assemblies in the presence of Al^{3+} for the complexed species formed as well as the species distribution diagrams.

Acknowledgements:
National sponsor agencies of Brasil – CNPq and Capes – and Universidade Federal do Paraná.

SESSION 1. ALUMINIUM CHEMISTRY

Poster presentations

Poster 1

Interaction of trivalent ions with various Aluminium minerals

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We studied the interaction of trivalent ions with a number of Aluminium mineral particles (gibbsite, bayerite, corundum) by time resolved laser fluorescence spectroscopy (TRLFS) and batch adsorption experiments with Cm and Eu. The spectroscopic data and pH dependent uptake results show distinct differences between the different surfaces. Adsorption on bayerite and corundum starts at lower pH than on gibbsite. This may be explained by the relatively high isoelectric point (IEP) found for our gibbsite (pH 11, compared to IEPs of below pH 10 for the other two solids). Also the TRLFS results suggest that one surface species that is found on bayerite and corundum at low pH is not formed on gibbsite. Finally, TRLFS results with gibbsite indicate the possible formation of a distinct "incorporated" surface species, which is not found under similar conditions in the other two systems. The presence of this species is crucially dependent on the experimental procedure, such as speed in pH increase and original state of the gibbsite suspension. We are currently trying to understand the origin of the "incorporated" species in the gibbsite system by new sets of experiments.

Poster 2

Evaluation of hydroxypyridinecarboxylic acids as new possible chelating agents for aluminium: solution chemistry, cytotoxicity, octanol/water partitioning, and chelation efficiency

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Chelation therapy is the most efficient therapeutic approach for metal ion overload. The chelators presently used for Al overload therapies, desferal and deferiprone, have several drawbacks. A multidisciplinary search for alternative molecules is being actively pursued. We proposed some hydroxy-pyridine-carboxylic acids (HP) (and references therein) as potential chelating agents for Al, as they have several requirements for an ideal chelator. They have negligible or low toxicity, high stability of the Al(III) complexes at physiological conditions, low affinity towards essential metal ions to reduce undesired metal depletion, low molecular mass (less than 400 Dalton) to allow oral administration, no redox activity *in vivo*, and their Al(III) complex at physiological pH are hydrophilic so to enhance metal ion urinary elimination. In the present poster, the following results of several HP derivatives will be reported: Al(III)/HP solution chemistry, cytotoxicity, octanol/water partitioning, and chelation efficiency.

Poster 3

Computational studies on surface complexation of aluminum nanoparticles with biomolecules

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With the rising of nano science and technology, there is a great concern on the impact of nanometer-size particles to the environmental and biological systems. Particularly, the polyoxoaluminum species, such as Al_{13} and Al_{30} clusters with Keggin structures, have been found in environmental samples. Spectroscopic and other experimental methods were employed in the studies of the interactions between the aluminum hydroxide clusters and biomolecules. However, it remains unclear that what is the exact nature of the surface complexation sites on the clusters, and how the size of the nanoparticules affects the complexation interactions. With the employment of electronic structure calculation and molecular dynamics simulation, we aim to provide the clues for these questions. Density functional theory is used to investigate the different surface complexation models and to obtain the tentative interaction parameters. Molecular dynamics simulation is then called for examining the impact of the clusters to the conformations of some biomolecules.

SESSION 2. ALUMINIUM GEOCHEMISTRY

Platform presentations

Platform 4

Aluminium export from acidified, nitrogen-saturated forest soils to waters

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Abstract: We evaluated factors controlling long-term and seasonal trends in aluminium (Al) export from two mountain catchments in the Bohemian Forest, Czech Republic. Soils in both catchments are strongly acidified (pH_{CaCl2} <4.5, base saturation <15%) due to high atmospheric deposition of sulphur (S) and inorganic nitrogen (N), and are N-saturated. Al is exported from soils to fresh waters mostly in ionic (Al_i) and organically-bound (Al_o) forms. The Al_o export is tightly associated with export of dissolved organic carbon (DOC), whereas that of Al_i with elevated leaching of strong acid anions (sulphate and nitrate). The Al export was dominated by Al_o in the pre-acidification period (<1850). During the acidification phase (1850-1990), contribution of Al_i to the total terrestrial Al export continuously increased, being mostly associated with sulphate. The proportion of Al_i associated with nitrate leaching increased in the ~1970s due to N-saturation of the catchments. Currently, after a drop in S deposition, nitrate has become the dominant strong acid anion and the major Al_i carrier.

Platform 5

High concentrations of inorganic aluminum in streamwaters were observed in catchments covered by old Spruce Stands

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Upper plateau of Jizera Mountains in the northern Czech Republic was significantly acidified during second half of 20th century by deposition of sulfur and nitrogen from powerplants in adjacent coal basins in the Czech Republic and Poland. As a result, many of spruce forests died during 1980´s and streamwater in the region were significantly acidified. At present, significant recovery was observed in catchments where forest died or has been harvested. Statistically significant negative trend (n= 20, r^2= 0,50, P<0.001) was observed between concentration of toxic inorganic Al (Al_i) and the percentage of young (<20 years) spruce stands within the catchments.

Elevated Al_i was probably enhanced by dry deposition of S and N onto canopy of mature trees. At the present, throughfall deposition under the spruce canopy is 20-30 $kg^{-1}ha^{-1}year^{-1}$ and deposition in open field 8-10 $kg^{-1}ha^{-1}year^{-1}$. Deforested catchments (now covered by young spruce plantations) thus received during last 20 years significantly lower acidic load in comparison with forested catchments. Soils in forested catchments were more acidified and leaching of Al_i was enhanced.

SESSION 2. ALUMINIUM GEOCHEMISTRY

Poster presentations

Poster 4

The new method for speciation analysis of aluminium in soil samples by hyphenated technique HPLC/IC-FAAS

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The research presents a new method for determination of inorganic speciation forms of aluminium: AlF_x, and Al^{3+} by means of the HPLC/IC-FAAS. The separation of Al species with nominal charge of +1, +2, +3 required a run time of less than 5 minutes during a single analysis. The proposed method has been successfully used for speciation analysis (qualitatively and quantitatively) of inorganic aluminium forms $AlF_n^{(3-n)+}$ in soil samples. The results of model studies were conducted in order to define the speciation distribution of soil samples in water extracts. Concentration determinations of particular aluminium forms were performed based on model studies and real samples. The separation of speciation aluminium forms occurred in the chromatographic column CS5A of HPLC, and then the online eluat was directed to F-AAS nebulizer. The studies of model solutions enabled the separation of the following aluminium forms: AlF_2^+, AlF^{2+} i Al^{3+}. Based on the analysis of water extracts of soil, an analytical signal occuring before form AlF_2^+ was obtained. In order to determine these forms, which elute before AlF_2^+ , a simulation using the Mineql program was conducted. The forms are AlF_4^- and AlF_3^0, and they were not observed in the model studies. The concentration of selected cations and anions was determined using ion chromatography.
It should be underlined that the studies of both model solutions and real samples, as the initial studies, enable a deeper insight into the forming processes of aluminium-fluoride complexes and, further, into the mechanisms accompanying these processes.

The research was supported by the Polish Ministry of Science and Higher Education through research grants No. N 305 101235, N 305 362833, N305 497234

Poster 5

Silicon solubility control in curative mineral waters, the Sudetes Mts., SW Poland. Role of aluminosilicate solid phases

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The chemistry of curative waters from all spas in the Sudetes, SW Poland, has been interpreted in terms of the solubility of solids for indicating phases which may be responsible for controlling the silicon activity. Silicon is, traditionally, recognized as a component giving therapeutic properties for curative-medicinal waters. Sudetic groundwater used in balneotherapy can be divided on two types: (1) low-enthalpy CO_2-rich water with various cationic composition (mainly Ca, Mg, Na, Fe), and (2) thermal sulphate or bicarbonate waters, usually fluoride-rich and containing H_2S and/or Rn.

Evaluation of hydroxyaluminosilicate colloid (HAS_B) solubility in waters by using the method proposed by Schneider et al. (2004) indicates that equilibrium with HAS_B can be maintained in thermal waters, with pH above 6.5. Interpretation of aqueous chemistry suggests that in both, cold and thermal waters silicon might be controlled by partial chemical equilibrium of incongruent reaction between aluminosilicate phase (halloysite) and $Al(OH)_3$ forms, and/or by reversible congruent dissolution of SiO_2 forms (like chalcedony), in cold water especially. The role of incongruent reaction between the HAS_B colloid and $Al(OH)_3$ forms is more likely in thermal waters.

Poster 6

Study of interactions of relevant organic acids and aluminium in model solutions using HPLC and IC

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The interactions of different organic acids such as citric, malic, oxalic and fulvic with aluminium were studied using ionic exchange chromatography and reversed phase HPLC. Detection of separated Al-organic complexes was performed by post-column derivatization using UV/VIS detection.
The experiments were carried out at low pH (1.5 – 3.1) with different concentration of Al ($7.41x10^{-5}$M - $7.41x10^{-4}$M) and solutions of various organic acids ($0.1x10^{-3}$M - $100x10^{-3}$M). The results were compared with model chemical equilibrium calculations.
The strongest effect observed was with oxalic acid and fulvic acid on the speciation of Al. These two ligands formed more than one type of complex with Al. In contrast there was no significant effect of malic acid on Al speciation and a rather weak effect caused by citric acid.
The studies show that the complexes formed are stable even at a low pH.
Therefore, organic acids can form different types of Al complexes with different stabilities and chromatographic characteristics. Such studies may help to understand the nature of organic acid complexes that occur with Al in vivo.

SESSION 3. ALUMINIUM AND PLANTS

Platform presentations

Platform 6

Simulation modeling in an effort to account for disparate electrical and ion-flux effects of Al upon plant cells

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When Al is introduced into solutions bathing plant tissues and cells, multiple electrical and ion-flux effects are observed. These effects include depolarization of the cell wall and the cell-membrane surface, enhancement or reduction of the transmembrane potential difference, and inhibition or enhancement of ion fluxes through channels and pumps. Some of these effects, *e.g.*, Al-induced transmembrane depolarization, may initiate toxicity resistance mechanisms or simply be a response to them. We are now in a position to apply quantitative, heuristic models to the observed data. For example, the Goldman-Hodgkin-Katz equation, when modified to incorporate cell-surface electrical potentials, may account for saturation, *cis*- and *trans*-inhibition, rectification, voltage gating, shifts in voltage optima, and other phenomena. These models, using best-available parameter values, should help to interpret observed responses and to focus attention upon unexplained responses.

Platform 7

Plasma membrane fluidity is the target of aluminum ions in plants

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Aluminum (Al) toxicity is the main limiting factor in crop production in areas with acid soils. The first symptom of Al toxicity is the root growth inhibition within minutes of exposure. The primary cause of root growth inhibition is not known. To identify the first target Al in roots, we studied the Al toxicity in *Arabidopsis thaliana* (*A.t.*) roots. Root growth was inhibited within first 2 minutes of exposure. Al induced almost immediate plasma membrane depolarization followed by the inhibition of endocytosis. Using plasma membrane isolated from BY-2 tobacco cells we have showed by spectrofluorometric measurements with laurdan that Al decreased plasma membrane fluidity. Al-induced *A.t.* root growth inhibition was partially reversed by membrane fluidizer benzyl alcohol supporting the role of membrane fluidity in Al toxicity.
Altogether, our data show that the membrane fluidity decrease is one of the main toxic effects of Al leading to rapid root growth inhibition.
Support: 522/05/0340; LC06034; MSM 0021620858.

Platform 8

Aluminium toxicity and plant signal transduction in cell suspensions of *Coffea arabica*

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Aluminium (Al) is the most abundant metal on Earth's crust (7% of the all elements). Toxicity due to this metal is widely documented in tropical acid mineral soils and is the major factor limiting over the productivity of crop species. Coffee is one of the most important crops economically worldwide. This crop grows on acid soils where the availability of Al is greater; therefore coffee yield is limited by the toxic effects of this element. We have develop a biological model in which suspension cells of *Coffea arabica* have been used. We found that aluminium toxicity affect the activity of different enzymes involved in the metabolism of phosphoinositides. We also are looking for the effect of phosphate and salicylic acid and their relationship with aluminium toxicity on this signal transduction mechanism. An overview of the latest results will be presented.
Research founded by CONACYT (45798-Z).

Platform 9

Distinctive - constitutive and aluminium-induced - patterns of phenolic compounds in two maize varieties differing in aluminium tolerance

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In maize several Al tolerance mechanisms seem to operate. Besides exclusion, internal detoxification could be responsible. Here we report on the profile of soluble phenolics in both roots and root exudates from two maize varieties differing in Al resistance. Under control conditions roots of Al-tolerant Cateto were characterized by higher concentrations of catechol, cumaric, ferulic and caffeic acids than Al sensitive var. 16x36. In Cateto, Al enhanced root levels of cumaric acid, and of taxifolin, while levels of caffeic and ferulic acids were decreased. In var.16x36, root concentrations of soluble phenolics were hardly affected and taxifolin levels remained clearly below those of Cateto. Cateto also had substantially higher concentrations of soluble cumaric, ferulic and caffeic acids in the root tips. Aluminium reduced the constitutive exudation of cumaric and ferulic acids from roots of both varieties. Our results suggest that Al tolerance in Cateto is related to a shift from phenylpropanoid pathway into flavonoid synthesis. Taxifolin may play a role both as an Al ligand and as an antioxidant.

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SESSION 3. ALUMINIUM AND PLANTS

Poster presentations

Poster 7

Influence of Al^{3+} on membrane bioelectrical parameters of Nitellopsis obtusa cell

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The first reactions of organisms to toxic compounds take place at the molecular and cellular level, before the effects become visible at higher levels of biological organization. Some responses of plant cells to Aluminium are related to the alteration of plasma membrane properties. The bioelectrical response of cell membrane is one of the most suitable and rapid methods for investigation of membrane functionality and transport systems. In plants, as well as animals, an adaptive response to environmental conditions and disturbances can be mediated by changes in the flow of ions across cell membranes. In some plants, as in animals, this takes the form of an action potential (AP). Cells of charophytes are well- explored experimental systems to study a wide range of membrane transport phenomena and cell separated from talloma can be considered to be a single organism that maintains essential physiological characteristics for a long time. Characeae *Nitellopsis obtusa* is very useful model system because of the ability of internodal cells to survive after isolation from the plant, and the large size (length about 10 cm, diameter up to 1 mm), regular shape of these cells and the ability to generate action potentials. It therefore provides an ideal model system for study of the various possible effects of aluminium on membrane bioelectricity. Charophyte algae can be characterized by a very negative membrane potential – up to -250 mV and more. The strongly negative membrane potential affects the driving force for any passive ion movement. The resting membrane potential of plant cells is composed of two components – the passive diffusion potential and the active potential generated by the electrogenic proton pump. Action potentials involve a transient influx of Ca^{2+} to the cytoplasm, effluxes of K^+ and Cl^-. The depolarization phase of AP is caused by an influx of Cl^-. Action potential repolarization comprised two stages. Repolarization phase is due to the outward K^+ flow and the activity of the electrogenic pump at the plasma membrane. It is supposed that the second stage of repolarization during AP is related to the operation of electrogenic H^+-pump in the excitable membrane. K^+ efflux is controlled by calcium- dependent potassium channels, which close when the Ca^{2+}_{cyt} decreases.

Al^{3+} has the potential to affect membrane bioelectricity by directly interacting with the membrane or by affecting channels and H^+-pump. There is the possibility that the environmental pH per se may influence depolarization of the membrane potential. The effect of aluminium on membrane potential and the generation of action potential were examined by comparing membrane potential dynamics and shape of AP before and after treatment with Al in various pH solutions. Aluminium treatment was carried out in the basic solution (APW), containing 0.1 mM KCl, 1.0 mM NaCl, 0.1 mM $CaCl_2$, 5mM TRIS, supplemented with 1 mM $AlCl_3$ at pH 4.2, pH 5.6, and pH 7.2 adjusted by HEPES and HCl.

The conventional glass-microelectrode technique was used to registrate electrical characteristics of internodal *Nitellopsis obtusa* cells. Signals were amplified with a WPI DAM50 preamplifier (input impedance - 10^{12} Ω, input leakage current - 50pA, gain – 20x). Action potentials were elicited by injecting 0,1 s duration square pulse over threshold depolarizing current (1μA/ cm^2)

between two pools using Ag/AgCl wires. Data were A/D converted (12 bits, ADS7806P, Burr-Brown Corporation) and stored in the computer memory for later analysis of membrane parameters.

We found that the effect of aluminium on membrane potential depends on pH. We observed statistically significant depolarization of membrane potential from –219 mV, to –160 mV at pH 4.2.

Amplitude of action potential depended on pH per se, but Al^{3+} attenuated it value in 66mV. We could conclude that Al^{3+}, but not pH affect electrical characteristics of internodal *Nitellopsis obtusa* cells.

Poster 8

Studies on the Aluminium (Al) absorption in protoplasts isolated from *C. arabica* L. suspension cells

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Coffee in México is the most important crop to export and *C. arabica* L. plants growth mainly in acid soils promoting aluminium (Al) toxicity as a negative factor on this crop productivity. Al- toxic effect is the root growth inhibition and has been identified also in *C. arabica* L. suspension cells. This inhibition is related with the Al-plant cells association, specifically with the cell wall and plasma membrane. To understand the mechanism of Al action in the cell metabolism we will use protoplast obtained from *C. arabica* L. suspension cells to evaluate the kinetics of Al-absorption. Also we are using fluorescent tracers (Morin and Calcofluor) in order to obtain the location and identify of the internal structures where Al interact.

This work is supported by CONACyT grant 45798-Z and a fellowship (4422) of the National System of Researchers to ERB.

SESSION 4. ALUMINIUM AND THE ENVIRONMENT

Platform presentations

Platform 10

Tissue accumulation of aluminium cannot be used to predict its toxicity in *Lymnaea stagnalis*.

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The level of tissue accumulation of a toxic metal is often taken as an indicator of its potential toxicity. The freshwater snail *Lymnaea stagnalis* were exposed to Al (500µg l^{-1}) alone or in the presence of different ligands (either a fulvic acid surrogate (FAS; 10mg l^{-1}) or phosphate (500µg l^{-1} P)) that were predicted to have different effects on the lability, and hence bioavailability, of Al. Behavioural toxicity over 30 days exposure was assessed, and tissue accumulation of Al quantified. FAS increased both lability and observed behavioural toxicity, whereas phosphate decreased lability and abolished the Al-induced toxicity. More Al accumulated in the tissues of snails exposed to Al+P compared to those exposed to Al alone ($p<0.05$), whereas there was less Al accumulation in the snails exposed to Al+FAS. These findings demonstrate that the degree of tissue accumulation of aluminium cannot be used to predict its potential toxic effects.

Platform 11

Mobilisation of river transported colloidal aluminium in estuaries and the impact on migrating Atlantic salmon smolts (*Salmo salar* L.)

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The liming of acid rivers in Norway has increased salmon production. In some rivers entering estuarine fjords, however, salmon return rates are low compared to what is expected from the number of fish (smolt) migrating from the rivers spring. Based on *in situ* Al fractionation of waters and sampling of gills from exposed smolt, results demonstrated that Al-species were mobilised from colloidal river transported material and formed low molecular mass (LMM) Al-species accumulating on gills in the estuaries. In estuarine surface water with 2-10 in salinity the concentration of LMM Al-species and Al accumulation on gills were significantly higher than in the input river water. Since smolt equipped with acoustic tags indicated unormal low swim velocity in part of the fjord with such estuarine waters in 2007 and 2008, the mobilisation of Al in brackish water may have an essential role affecting the migrating and hence return rates of adult salmon.

SESSION 4. ALUMINIUM AND THE ENVIRONMENT

Poster presentations

Poster 9

Toxicity of aluminium originating from zeolite A on the *Acinetobacter junii*

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The antibacterial effect of commercial zeolite A against the pure culture of phosphate-accumulating bacterium *Acinetobacter junii* was investigated. The estimated EC_{50} values of the zeolite for the inhibition of bacterial colony forming units was 0.33-0.37 g/L by investigated initial pH values of 6, 7 and 8. The toxic effect of zeolite is ascribed to the generation of Al (0.38-1.09 $x10^{-5}$ g/L) and Si (2.02-2.93 $x10^{-5}$ g/L) ionic species during the process of hydrolysis and dissolution of zeolite (leaching of Al, Si and Na). The toxic effect of Al was confirmed in separate experiments, where the aluminum toxicity (in the form of aluminum sulphate) was estimated as EC_{50} of 3.45-3.89 x 10^{-4} g/L. These results suggest the importance of controlling the amounts of synthetic zeolites which can be released to different compartments of environment and accumulate there as nondegradable material. Zeolite A may be the important source of Al in influent of the biological wastewater treatment systems, where they can cause unwanted antibacterial effects.

Poster 10

Fate and behaviour of aluminium used in the lake restoration.

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The chemical treatment of lake sediments with Al salts is a common practice in the lake restoration, nevertheless the understanding of its full ecological effects is incomplete. During the restoration of the eutrophic hardwaterlake Tiefwarensee (1.41 km², mean depth 9.6 m, maximum depth 23 m), Mecklenburg-Vorpommern, Germany, from 2001 to 2005 by hypolimnetic addition of $Al(OH)_4$ and $Ca(OH)_2$ the nutrients monitoring was accompanied by Al analysis to assess the risk of Al toxicity on aquatic organisms. The concentrations of acid reactive Al species in the water reached in maximum 2 mg L^{-1} in parts of the anoxic hypolimnion immediately during the two weeks lasting cycles of the Al addition. Perchs investigated before and during an Al treatment cycle in summer 2003 showed a significant Al accumulation on gills (100 µg Al g^{-1} dw) during the Al addition whereas roachs, breams, and silver carps stayed unaffected. An Al balance two years after the treatment indicates the complete export of the added Al into the sediment.

Poster 11

New development in the analysis of aluminium fluoride complexes using HPLC coupled with F-AAS detection

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In this work, a new application for the separation of the aluminium fluoride complexes AlF_2^+, $AlF^{2+,}$ and $Al^{3+}$of water samples by hyphenated technique HPLC-FAAS is presented. Seperation of inorganic aluminum species was achieved using IonPac CS5 (Dionex) analytical column with guard column IonPac CG 5 (Dionex) and an aqueous ammonium chloride mobile phase, at pH 3.00 with gradient elution. The study of the behavior of the analytical columns used in the separation of AlF_x forms in the presence of NH_4Cl, K_2SO_4, Na_2SO_4, $(NH_4)_2SO_4$, NH_4NO_3 as the eluents were examined. Besides the type of analytical columns were also investigated for its selectivity in resolution of inorganic aluminium species. The optimized method allowed to get full separation of three forms: AlF_2^+, $AlF^{2+,}$ and Al^{3+} during one analysis in tap water and ground water samples, without using post-column reaction. The obtained results provide valuable information necessary for an assessment of a potential threat posed by toxic forms of aluminium to aquatic ecosystems.
In this presentation, after describing the experimental setups, the above mentioned examples of separations will be presented in detail.

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SESSION 5. ALUMINIUM AND BIOCHEMISTRY

Platform presentations

Platform 12

Speciation of Al in biological samples

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Al is associated with many clinical disorders in renal patients. Al accumulation in brain has also been related to the neurodegenerative processes in Alzheimer's disease. In order to better understand Al transport in the human body, it is necessary to know the chemical species in which Al is present in body fluids and tissues. Among a variety of biological samples, Al speciation was frequently investigated in human serum. Progress was made in the development of analytical techniques for the determination of the amount and composition of high molecular mass Al (HMM-Al) as well as low molecular mass Al (LMM-Al) species in human serum. However, due to the complex chemistry of Al in serum, its low total concentration and the high risk of contamination by extraneous Al, speciation of Al in biological fluids is still a difficult task for analytical chemists. In the present work the problems related to Al speciation in human serum are discussed. The potential of the use of different analytical approaches for Al speciation is critically evaluated in order to propose analytical protocols for reliable speciation of Al in serum at low $\mu g\ L^{-1}$ concentration range.

Platform 13

Combination of CIM® monolithic chromatography with UV and ICP-MS detection for speciation of Al in human serum

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In speciation analysis it is important to apply and compare complementary analytical procedures to obtain reliable analytical data. As an alternative to FPLC columns containing ion-exchange resins, ion-exchange separation supports based on CIM® (Convective Interaction Media) were developed in the last decade. The matrix supports made of highly porous poly(glycidyl methacrylate-co-ethylene dimethacrylate) supported with strong or weak cation and anion exchangers offer very fast separation of biomolecules. CIM® monolithic chromatography in combination with ICP-MS detection can offer unique analytical tools for speciation analysis at very low concentrations in spiked human serum. Furthermore, CIM® monolithic columns enable speciation of Al in unspiked human serum. Monolithic column bearing DEAE (diethylamino-ethyl) functional group with UV and ICP-MS detection was applied for speciation of HMM-Al compounds in unspiked serum of a renal patient. Separated Al species were detected »on-line« by ICP-MS. Well-resolved protein peaks were obtained. Data indicated that 93 ± 7% of Al in unspiked serum of a renal patient was eluted exclusively under the transferrin peak. Transferrin was identified on the basis of the retention volume and also by the capillary nano liquid chromatography electrospray ionization mass spectrometry (cap nano LC ESI-MS).

Platform 14

Aluminum toxicity and energy production in human astrocytoma cells: molecular clues to aluminum-induced neurological diseases

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Although aluminum (Al), a known environmental toxin, is thought to be involved in the etiology of a variety of neurological diseases, its precise molecular interaction within the brain is not completely understood. Lactate is an important contributor to cerebral metabolism; however its exact role in the brain energy budget is yet to be fully delineated. Using human astrocytes as a model system, we have uncovered how Al toxicity provokes the conversion of lactate into fatty acids. Various metabolomic and proteomic techniques have helped unravel the molecular mechanisms leading to enhanced lipogenesis in Al-challenged astrocytoma cells. While under control condition lactate was utilized in oxidative-ATP production via mitochondrial lactate dehydrogenase (mLDH), Al-stressed cells utilized lactate to generate pyruvate, a keto acid that mediates the detoxification of reactive oxygen species (ROS). The participation of pyruvate as a ROS scavenger resulted in the generation of acetate, a precursor to lipid synthesis. The increased activities of acetyl-CoA synthase (ACS) and acetyl-CoA carboxylase (ACC) orchestrated the deposition of lipids in the Al-exposed astrocytes. This study reveals the role of mLDH in anti-oxidative defence and provides molecular clues to the neurological abnormalities triggered by Al.

Platform 15

Fusion pore properties and aluminium in rat pituitary lactotrophs

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Exposure of workers to aluminium is associated with a reduced level of serum prolactin, a stress peptide hormone mainly synthesised and secreted by the anterior pituitary lactotrophs. The aim of this work was to study whether aluminium exposure affects secretory activity in cultured rat pituitary lactotrophs. For this we measured regulated exocytosis at elementary level by monitoring the properties of fusion pore, a membranous structure, which forms upon fusion of the vesicle membrane with the plasma membrane. We performed high-resolution patch-clamp measurements of membrane capacitance, a parameter related to membrane area fluctuations associated with the unitary fusion events of large dense-core vesicle with the plasma membrane, in spontaneous and stimulated conditions. After 24-h exposure to 30 μM $AlCl_3$, the exocytotic response to depolarizing stimulation was repressed and differences in the elementary fusion pore properties were detected. In the presence of aluminium, the fraction of events with a measurable pore conductance increased in comparison to non-treated controls. However, these fusion pores exhibited a pore diameter smaller than the molecular size of prolactin molecules. These results indicate that sublethal aluminium concentrations shift the mode of elementary exocytotic events towards release-incompetent ones, favouring fusion pores through which the large cargo molecules like prolactin can not pass.
This work was supported by the grants P3 310 381, Z3 7476 1683 and BI-PT/06-07-002 (The Ministry of Higher Education, Sciences and Technology of the Republic of Slovenia), 4.1.1 Eslovénia (The GRICES of the Portuguese Ministry of Sciences, Technology and High Education) and SFRH/BD/41217/2007 (The FCT of the Portuguese Ministry of Sciences, Technology and High Education).

Platform 16

Therapeutic potential of bak gene silence in aluminum induced neural cell degeneration

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The high potency of bak RNAi, as shown by many experimental studies, makes it a rational candidate co-therapeutic agent in neural cell degeneration. In the present study, cell degeneration model was established in human neuroblastoma cells treated by aluminum. In the cell model, we have selected and validated the most effective small interfering RNA sites of three predesigned siRNAs of bak gene by cell viability analysis, and confirmed which on gene level by qRT-PCR and on protein level by Immunocytochemical assay. Our data identified the site 1 siRNA as the most effective siRNA at the optimal transfective reagent at concentration of 10 mM and transfective course of 48 h. The efficiency of transfection rate and interference rate were 93% and 57.76%, which were closely correlated with the siRNA knockdown efficiency of human Bak protein, as well as with the upregulation of cell survival activity. Neuroblastoma cells with Bak knocked down showed a clear resistance against cell demise and aluminum induced apoptosis. These results indicate that the genetic inactivation of Bak in recombinant neuroblastoma cells can be an effective strategy in delaying the onset of apoptosis in fed-aluminum cultures. The present study further supports the therapeutic potential of RNAi-based methods for the treatment of neurodegenerative diseases.

Platform 17

New insight on DNA topological changes induced by aluminum: Relevance to Alzheimer's disease

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Alzheimer's disease (AD) is one of the devastating progressive neurodegenerative disorders and associated with excessive deposition of Al in the region of the brain that undergoes degeneration. Earlier our lab demonstrated presence of a left handed Z DNA conformation in the hippocampus and localization of Al in the chromatin region of AD brain. Based on this finding we hypothesize that metals like Al play a pivotal role in modulation DNA topology in AD brain. It is interesting to know whether helical change in the AD brain is due to accumulation of Al alone or effects of its secondary consequence. Keeping this in view and to investigate the above mentioned hypothesize experimentally, in our in-vitro studies we focused on the interaction of Al [$Al(maltol)_3$] with pUC 19 supercoiled(sc) DNA, genomic calf thymus(ct) DNA, single and double stranded circular DNA. The circular dichroism results showed that Al induce B-C-A mixed and Z–DNA conformation in scDNA and ctDNA respectively. Further nick translation and agarose gel studies indicated that Al caused double stranded breaks. These in vitro observations suggest that accumulation of Al in the AD brain could have an important and significant contribution to DNA topological (helicity and stability) change and damage associated with AD.

SESSION 5. ALUMINIUM AND BIOCHEMISTRY

Poster presentations

Poster12

Evaluation of cell viability in the presence of aluminium in rat pituitary lactotroph cultures

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Aluminium has been shown to accumulate in the cytoplasm of some cells in the anterior pituitary. The aim of this work was to study whether aluminium affects cell viability of rat pituitary lactotroph-enriched cultures. Dual-labelling flow cytometric assays were performed to quantify live and dead cells simultaneously. Following a 30 min-exposure of cell cultures to 300 μM $AlCl_3$ we observed a significant decrease (48 %) in cell viability in comparison to non-treated controls. At a ten-fold lower concentration, overt cell loss was achieved only by prolonging the cell exposure time to 4 days. Continuous exposure to $AlCl_3$ concentrations <30 μM, even for up to 4 days, resulted in unimpaired cell viability. These results indicate that aluminium may induce cell loss in the cultures from the anterior pituitary.
This work was supported by the grants P3 310 381, Z3 7476 1683 and BI-PT/06-07-002 (The Ministry of Higher Education, Sciences and Technology of the Republic of Slovenia), 4.1.1 Eslovénia (The GRICES of the Portuguese Ministry of Sciences, Technology and High Education), SFRH/BD/41217/2007, SFRH/BD27467/2006 and SFRH/BPD/14677/2003 (The FCT of the Portuguese Ministry of Sciences, Technology and High Education).

Poster 13

Inhibition of glia apoptosis induced by aluminium as a potential therapeutic strategy in degenerative diseases

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There is a vast amount of evidence indicating that bax play a major role in the development, maintenance, and survival of neurons and neuron-supporting cells such as gliacytes. Although various treatments alleviate the symptoms of neurodegenerative diseases, none of them prevent or halt the neurodegenerative process. The high potency of bax siRNA, as shown by many experimental studies, makes it a rational candidate of co-therapeutic agents in neurodegenerative disease. In the present study, the corresponding double-stranded DNA was used to construct small interference RNA (siRNA). Then, the siRNAs were transfected into glioma cells with SuperFect transfection reagent. All the cells with siRNA were screened out using Cy3. Interference rate of bax gene was measured by qRT-PCR, the content of Bax protein was examined through Immunocytochemical assay and the change of cell death rate was determined with cytometry. Our data showed that the cell viability transfected by site 1 of siRNAs was more obviously upregulated, which began to increase on 24h, and reached the most significance on 72h. There was a time-dependent relationship between the transcript of bax gene and the reduction of Bax with RNAi action. Besides, the most effective inhibition of bax mRNA was transfected by siRNA at the final concentration of 20 nM after 72h of transfection. The transfection rate labled by CY3 was above 90%, the interference rate was 62.3%. Inhibition of the bax gene can decrease the levels of Bax protein and cell apoptosis as well. Taken together, Bax is essential for apoptosis induced by aluminum. Some sites of Bax mRNA, for example, the site 1, may serve as a new drug target and RNAi probably can be used for treatment of degenerative diseases.

Poster 14

Effect of Al(III) to the conformations of reduced and oxidised glutathione

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Glutathione is a ubiquitous tripeptide playing a number of vital roles in cell metabolism. Mainly existing in its reduced form, glutathione acts antioxidant and protects cells from toxins such as free radicals. Decreased glutathione levels have been associated with increased oxidative stress in various neurological disorders including Lou Gehrig's, Parkinson's and Alzheimer's diseases. At its concentration of approximately 5 mM in animal cells, the ligand shows competitive complexation ability with Al(III). We have employed potentiometric titrations, electrospray mass spectroscopy (ESI-MS), multi-nuclei magnetic resonance spectroscopic (NMR) techniques and two-dimensional ^{1}H-^{1}H NOESY NMR to investigate the complexation of Al(III) with both the reduced and oxided forms of glutathione and how the conformations being affected by the coordination. Furthermore, we use first-principles calculation approaches and molecular dynamics simulation to illustrate the possible binding modes and how the conformations of the ligand change. The results may provide us further insight on the toxicity of aluminum.

Poster 15

The effects of aluminum on neuron development of the hippocampus in neonatal rats

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Postnatal day 3 hippocampus neuron were treated with 0, 1, and 2 μg/ml aluminum (Al) for 14 days and 100 ng/ml of nerve growth factor (NGF) were added into treatment media at day 7. Results indicated that hippocampus neuron division and differentiation increased with Al exposure time. Concentrations of 1 μg/ml Al , but not 2 μg/ml Al , increased cell viability compared to control ($p<0.05$). Immunofluorescence staining suggested that developmental marker proteins, NMDAR1A and NMDAR2A/B, were distributed both in the neuron cell membrane and the cytoplasm. The protein expressions of NMDAR1A and NMDAR2A/B decreased with increasing Al dosage and exposure time ($p<0.05$). Furthermore, 2 μg/ml of Al induced cell dissolution and apoptosis. Addition of NGF from the first day of incubation did not prevent Al induced decrease in NMDAR1A and NMDAR2A/B.

SESSION 6. ANIMAL MODELS OF ALUMINIUM TOXICITY

Platform presentations

Platform 18

Up-regulation in the expression of renin gene by the influence of aluminum

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Aluminum is excreted rapidly into urine through kidneys. After taking excess amount of aluminum such as aluminum-laden drugs, however, aluminum stays in longer periods in the bodies. The gene-expression after injection of aluminum was widely screened by differential display analysis in mice kidneys. Thirteen bands were altered in the animals with aluminum dose, 8 genes were up-regulated and 5 genes were down-regulated. The gene sequencing method positively identified only one of the up regulated genes, as renin (Ren1), and none of the down regulated genes. It was confirmed to increase the renin production in response to relatively low dose of Al in further experiments such as RT-PCR and Western blotting. It is pointed out that a chronic exposure of Al may be a cause of essential hypertension due to the up-regulation of renin.

Platform 19

Aluminum hydroxide and gulf war ALS: An *in vivo* model of motor neuron death

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Gulf War Illness (GWI) is a multi-system disorder afflicting large numbers of Coalition veterans of the first Gulf War. The disorder can include fibromyalgia, PTSD, chronic fatigue syndrome, and may show neurological deficits including amyotrophic lateral sclerosis (ALS). This ALS "cluster" represents the second such cluster described to date. Possible etiologies for GWI-ALS include adjuvants contained in the anthrax vaccine series, especially aluminum hydroxide. We examined the role of Al in young, male outbred CD-1mice injected *s.c* at an equivalent-to-human dose administered twice within a two week interval. After sacrifice, spinal cord/motor cortex samples were examined by IHC. Aluminum-treated mice showed significantly increased apoptosis of motor neurons and increases in reactive astrocytes and microglia. Morin stain detected the presence of aluminum in the cytoplasm of motor neurons with some neurons also positive for the presence of hyperphosphorylated tau. These data support a neurotoxic role for Al in GWI-ALS.

Platform 20

Amyloid precursor protein, acetylcholine metabolism, and ApoE in an aluminum-based rat model for AD

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A rat model for Alzheimer disease (AD) was exposed to human-relevant dietary aluminum levels throughout their middle age and old age. Outbred Wistar rats were trained to perform a hippocampal-dependent continuous alternation T-maze task. 0% that consumed the lowest aluminum dose, 20% the intermediate dose, and 70% that consumed aluminum at the high end of the human dietary aluminum range showed significantly lower mean scores for this test in middle age than in old age. The latter group had more aluminum accumulation in entorhinal and temporal cortical neurons, accompanied by cell shrinkage, shriveling of their processes, and synapse breakdown. A panel of immunocytochemical stains and a DNA analysis were carried out on the rat tissues in order to examine for difference between those that became cognitively-damaged and others that remained cognitively-intact. Markers of oxidative damage and hyperphosphorylated tau increased with aluminum accumulation in neurons. Markers for microtubules and synapses decreased in the cognitively-damaged rat brains. Here, antibody markers were used to investigate the rat brains for amyloid precursor protein (APP) and choline acetyltransferase (ChAT), the enzyme that catalyzes the synthesis of acetylcholine from its precursors, acetyl co-A and choline. Firstly, the immunostain for APP demonstrated a striking up-regulation of APP in the perinuclear cytoplasm, suggestive of a Golgi location, in neurons of the cognitively-damaged rats. In humans affected by Alzheimer disease, up-regulation of APP synthesis is an early stage of amyloid plaque formation. Secondly, the antibody marker for ChAT immunostained striatal tissue from cognitively-intact rats robustly whereas the striatum from cognitively-damaged rats stained poorly, if at all. In AD, ChAT activity declines as much as 70% (Hansen et al., 1988). Thirdly, as ApoE4 is a risk factor for AD, ApoE DNA was isolated from cells of both rat groups and sequenced to determine whether there was any ApoE difference between the two rat groups. The rats in both groups were found to have the same genotype and this was identical to the ApoE sequence reported for wild-type rats. Hence, this was not a distinguishing feature between the two groups. During the genotype analysis of the rat ApoE, it was noted that rat ApoE has arginine amino acids at both positions 112 and 158 as in the human ApoE4 allele. On the other hand, human ApoE2 and ApoE3 have different amino acids at one or both of these positions. Human ApoE4 is less capable than ApoE2 and ApoE3 of transporting cholesterol to synapses in need of repair. This commonality observed between human ApoE4 and rat ApoE structure may be an explanation for the reduction in neural plasticity and inability to respond to aluminum-induced neuronal damage. These observations may explain why outbred Wistar rats are responsive to cognitive change following chronic ingestion of aluminum at sub-pharmacological levels[1-3] and are thus an appropriate animal model for AD.

SESSION 6. ANIMAL MODELS OF ALUMINIUM TOXICITY

Poster presentations

Poster 16

Influence of thyroid hormones in aluminium's effect on intestinal calcium transport in a mammalian model

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Intestinal calcium absorption is an important process involved in maintenance of vertebrate calcium (Ca) homeostasis that is disturbed by aluminium (Al). It has been demonstrated that thyroid hormones, triiodothyronine (T3) and thyroxine (T4), can affect Ca metabolism both superimposing and interacting with classic hormonal regulation by calcitriol and parathormone at the level of small intestine. Here, we present a study to test the hypothesis that T3 and T4 might influence Al inhibitory effect upon intestinal Ca absorption. In orally short-term aluminium-treated rats with experimentally altered thyroid hormones levels, several aspects of intestine Ca transport were analyzed by using radioisotopic techniques (^{45}Ca) . Hyper- and hypothyroid conditions were achieved by means of administration of either exogenous T4 or methimazole (a T4 synthesis inhibitor). Results show that both Ca-uptake kinetic parameters and transcellular Ca flux across small intestine epithelium have certain degree of dependence on thyroid hormones serum level in Al-treated rats, preliminarily supporting our presumption.
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Poster 17

The effects of high aluminum load on oxidative status in gastrostomy tube-fed neonatal rats

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Three-day old neonatal rats were fed artificial rat's milk containing 0, 3, or 30 mg Al/L (as $AlCl_3$) by gastrostomy for 14 days. Results showed that body weight gain in high Al group (30 mg Al/L) rats were lower than the control group. The Al concentrations in plasma, liver and all brain tissues were higher in the high Al pups than the control and 3 mg Al/L group. In high Al group, medulla oblongata SOD activities, erythrocyte and hepatocyte CAT activities were significantly lower than the control group. Plasma GPx activity and TBARS concentrations in the plasma, liver, cerebral cortex, thalamus and hypothalamus tissues of the high Al group were also significantly higher. Therefore, dietary Al overload may increase oxidative stress in neonatal rats.

SESSION 7. HUMAN EXPOSURE TO ALUMINIUM

Platform presentations

Platform 21

An *in silico* model of aluminium binding by transferrin in serum

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We have used a systems biology approach to address the hitherto insoluble problem of the quantitative analysis of non-equilibrium binding of aqueous metal ions by competitive ligands in heterogeneous media. To-date, the relative proportions of different metal complexes in aqueous media has only been modelled at chemical equilibrium and there are no quantitative analyses of the approach to equilibrium. While these models have improved our understanding of how metals are used in biological systems they cannot account for the influence of kinetic factors in metal binding, transport and fate. Here we have modelled the binding of aluminium, Al(III), in blood serum by the iron transport protein transferring (Tf) as it is widely accepted that the biological fate of this non-essential metal is not adequately described by experiments, in vitro and in silico, which have consistently demonstrated that at equilibrium 90% of serum Al(III) is bound by Tf. We have coined this paradox 'the blood–aluminium problem' and herein applied a systems biology approach which utilised well-found assumptions to pare away the complexities of the problem such that it was defined by a comparatively simple set of computational rules and, importantly, its solution assumed significant predictive capabilities. Here we show that our novel computational model successfully described the binding of Al(III) by Tf both at equilibrium and as equilibrium for AlTf was approached. The model predicted significant non-equilibrium binding of Al by ligands in competition with Tf and, thereby, provided an explanation of why the distribution of Al(III) in the body cannot be adequately described by its binding and transport by Tf alone. Generically the model highlighted the significance of kinetic in addition to thermodynamic constraints in defining the fate of metal ions in biological systems.

Platform 22

Aluminium and congophilic amyloid angiopathy; the 200+ brains study

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In July 1988, 20 tonnes of aluminium sulphate was discharged by the South West Water Authority into the drinking water supplied to a large region of North Cornwall.
Up to 20 000 people were exposed to concentrations of aluminium which were 500–3000 times the acceptable limit under European Union legislation (0.200 mg/l). Although this incident is currently the topic of a government inquiry, nothing is known about its longer-term repercussions on human health. The first neuropathological examination of a person who was exposed and died of an unspecified neurological condition was carried out. A rare form of sporadic early-onset b amyloid angiopathy in cerebral cortical and leptomeningeal vessels, and in leptomeningeal vessels over the cerebellum was identified. In addition, high
concentrations of aluminium were found coincident with the severely affected regions of the cortex. Although the presence of aluminium is highly unlikely to be adventitious, determining its role in the observed neuropathology is impossible. A
clearer understanding of aluminium's role in this rare form of Alzheimer's related disease should be provided by future research on other people from the exposed population as well as similar neuropathologies in people within or outside
this group. The latter research is currently about to begin and we shall describe this research in this presentation.

Platform 23

Improving the non-invasive measurement of aluminium in bone

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Aluminium accumulation in humans can be toxic, so it is desirable to measure aluminium stores non invasively. Neutron activation promises to provide a measure of such stored aluminium.
Initial human data were obtained from measurements designed for manganese. So the timing was sub-optimal for ^{28}Al, which is produced by neutron activation and which has a half-life of 135 s. Even using 180 s irradiation time and a transfer between irradiation and counting of 105 s, the detection limit in humans was 28.0 μgAl/gCa and in standards it was 19.5 μgAl/gCa.
The timing has been reduced to 45 s irradiation and 30 s transfer. Also, the detection system has been upgraded. These improvements combined to reduce the detection limit in standards to 8.5 μgAl/gCa, which corresponds to 12.0 μgAl/gCa in humans. Expected concentrations for referent subjects are 20 - 30 μgAl/gCa, so the improved system should detect aluminium in most referent subjects.

SESSION 7. HUMAN EXPOSURE TO ALUMINIUM

Poster presentations

Poster 18

Effects of sex, age, and oral status on Mn, Cu, and Al concentrations in mixed saliva of elementary school children

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To examine the standard Al, Mn, and Cu concentrations in mixed saliva of children and the relationship between these levels and dental caries, sex, and age, 527 resting mixed saliva samples for Mn and Cu and 499 for Al were collected at an elementary school in Kitakyushu city at 10:00-11:30 a.m. in December 2004. The concentrations were determined by the calibration curve method using simultaneous multi-element atomic absorption spectrometry. The Al, Mn, and Cu levels were 0.2 ± 0.2 μg/ml, 22.0 ± 15.2 ng/ml, and 3.8 ± 4.1 ng/ml in the sound tooth group, respectively. The Cu level significantly increased with the presence of caries experience, and increased according to the number of untreated teeth. Mn levels were significantly higher in boys compared to girls and also higher in the upper grade compared to the lower grade. The Al concentration was not affected by such factors.

Poster 19

Towards a new diagnostic tool to measure bone aluminum levels

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We present *in vivo* measurements of aluminium (Al) stored in a hand bone in the population living in Southern Ontario, Canada. *In vivo* neutron activation analysis (IVNAA) proves to be suitable for measuring Al via the thermal neutron capture reaction $^{27}Al(n, \gamma)^{28}Al$. The photon spectra of the hand phantom closely resemble those collected from the hands of twenty-two subjects. The achieved minimum detectable limit of 0.29 mg Al or (19.5±1.5) μg Al/g Ca in the phantoms is within the range of the Al levels of 20 – 27 μg Al/g Ca found in other *in vivo* and *in vitro* studies. The mean Al level of the population is (27.1±16.1) μg Al/g Ca. The measurements required an equivalent dose of 17.6 mSv and an effective dose of 14.4 μSv. This diagnostic technique is available to measure patients with the documented overload of bone Al in the near future.

SESSION 8. ALUMINIUM, ALZHEIMER'S AND OTHER NEUROLOGICAL DISEASES

Platform presentations

Platform 24

Structural speciation studies in binary aqueous Al(III) systems with hydroxycarboxylate and phosphonate substrates

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Aluminum has since long time been recognized as a metallotoxin associated with numerous clinical pathologies, such as Alzheimer's disease, involving neurodegeneration. Prompted by the need to delve into the nature and physicochemical properties of well-defined aluminum species partaking of the chemical reactivity in requisite biological processes, structural speciation studies have been launched in our lab targeting: a) the synthesis and characterization of well-defined Al(III) binary species with low molecular mass substrates present in biological fluids and containing hydroxycarboxylate and phosphonate moieties, and b) aqueous solution studies delving into the speciation of the synthetically investigated binary Al(III)-systems. The collective studies support the nature and properties of the arising binary Al(III) species in the solid state and in solution, projecting well-defined Al(III) agents involved in biologically relevant interactions with sensitive targets. The overall chemistry reflects the tendency Al(III) to pursue further complex binary and ternary interactions with high molecular mass neuronal targets linked with Alzheimer's neurodegeneration.

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Platform 25

A critical review of the Al-literature aiming at the missing research strategies for the evaluation of neurotoxic risk of high Al-exposures in humans

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Highest human exposures to Al occur under normal conditions at industrial work-places, mainly by Al-inhalation (e.g. dust, welding fumes), leading to permanent internal Al loads up to 20 times higher (200 µg Al/l urine) than reference loads of the general population. The review shows, considering 40 neurobehavioral animal and 20 human studies, that the results of existing research models are not only controversial but also based on study designs not suited to evaluate the potential neurotoxic risk of high and long-term AL-exposure. Main shortcomings are missing data on internal Al-loads in animal studies (bioavailability) and missing control of potential intellectual 'a priori' differences (asymmetry) between exposed and non-exposed workers in cross-sectional studies. Two recent longitudinal studies did not reveal neurobehavioral changes (repeated measurements) in Al-exposed workers. It is proposed to change generally from mainly static to dynamic longitudinal approaches and to investigate systematically the interaction between Al-exposure and biological ageing (sensitivity) in sequential phases of lifetime. A corresponding model and data are shown.

Platform 26

Macrophagic myofasciitis-Associated Cognitive Dysfunction

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Background: Macrophagic myofasciitis (MMF) is an emerging condition, characterized by specific muscle lesions assessing long-term persistence of aluminum hydroxide within macrophages at the site of previous immunization. Affected patients mainly complain of arthromyalgias, chronic fatigue, and cognitive difficulties.
Objectives: This study was designed to characterize the MMF-associated cognitive dysfunction (MACD) and to assess its specificity.
Methods: Based on preliminary routine neuropsychological evaluation of 22 MMF patients, we designed a comprehensive battery of neuropsychological tests to prospectively delineate MACD. In a case-control study we included 11 consecutive patients with MMF at muscle biopsy and 11 control patients with arthritis and chronic pain. These controls, similar in age, educational level, pain, fatigue and depression, were used to distinguish specific from non-specific cognitive impairment. Then, we ran a cohort study including a total of 25 patients to characterize MACD and test clinical factors could influence its severity.
Results: Compared to controls, MMF patients showed pronounced specific cognitive impairment. In the cohort study, patients had measurable stereotyped cognitive dysfunction, mainly affecting (i) both visual and verbal memory; (ii) executive functions, including attention, working memory, and planning; and (iii) interhemispheric connexion. Cognitive deficits did not correlate with pain, fatigue, depression, or disease duration. They were suggestive of organic cortico-subcortical damage with deep white matter alterations. Pathophysiological mechanisms underlying MACD remain to be determined.
Conclusions: MMF-assessed long-term persistence of aluminum hydroxide within body was associated with cognitive dysfunction, and the association was still observed after adjusting for chronic pain, fatigue and depression.

Platform 27

Characterization of a miRNA-146a-mediated inflammatory circuit in Alzheimer's disease (AD) brain also up-regulated in aluminum-sulfate-stressed human brain cells

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Micro RNAs (miRNAs) consist of a family of small, 22 nucleotide long ribonucleic acids that are post-transcriptional regulators of messenger RNA (mRNA) complexity. In mammalian systems, to date about 856 miRNAs have been identified and characterized, however, the brain appears to utilize only about one quarter of these in the regulation of normal gene expression functions. Human brains thereby retain discrete populations of miRNA species that support physiologically homeostatic gene expression patterns, however, certain miRNA abundances are significantly altered in normal aging and in neurological disorders such as Alzheimer's disease (AD). Here we provide evidence in AD brain, versus age-matched controls, of a specific up-regulation of an NF-κB-sensitive miRNA-146a that targets and down-regulates the expression of complement factor H (CFH), an important repressor of the brain's inflammatory response. Transfection of human neural (HN) cells in primary culture using an NF-κB-containing pre-miRNA-146a promoter and luciferase reporter in interleukin-1β plus Aβ42 peptide (IL-1β+Aβ42) stressed HN cells showed significant up-regulation of miRNA-146a signaling that corresponded to decreases in CFH gene expression. Treatment of stressed HN cells with the metal chelator, anti-oxidant and NF-κB translocation inhibitor pyrollidine dithiocarbamate (PDTC), or the resveratrol analog CAY10512, effectively abrogated this response. Incubation of an antisense oligonucleotide to miRNA-146a (anti-miRNA-146a; AM146a) was found to restore CFH expression levels. Incubation of aluminum sulfate or aluminum sulfate plus iron sulfate together in HN cells at nanomolar concentrations emulated these NF-κB-mediated inflammatory signaling events. These data suggest that NF-κB-sensitive, miRNA-146a-mediated, modulation of CFH gene expression may in part regulate the inflammatory response in stressed HN cell models and in AD brain, and underscores the potential of aluminum to contribute to pathogenic and genotoxic mechanisms known to contribute to the AD process.

Platform 28

Demonstration of aluminium in the brains of patients with Alzheimer's disease

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Brains (hippocampus and temporal lobe) of patients with Alzheimer's disease were examined by energy-dispersive X-ray spectroscopy combined with transmission electron microscopy (EDX-TEM) and by secondary ion mass spectrometry (SIMS). EDX-TEM analysis allows simultaneous imaging of subcellular structures with high spatial resolution and analysis of small quantities of elements contained in the same subcellular structures.
By EDX-TEM analysis, we demonstrated the presence of Al in amyloid fibers located in senile plaques in brains of patients with this disease. Al was also detected in the nuclei of nerve cells and non-neural cells by EDX-TEM and SIMS analyses. High concentrations of Al were demonstrated in the nucleoli and heterochromatin regions of nerve cells by EDX-TEM analysis. The possible role of Al in the pathogenesis of Alzheimer's disease is discussed.

SESSION 8. ALUMINIUM, ALZHEIMER'S AND OTHER NEUROLOGICAL DISEASES

Poster presentations

Poster 20

Interactions between Al(III) and phosphonate-carboxylate substrates relevant to Alzheimer's DISEASE

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Aluminum is a metal ion present in the earth's crust. Previous studies have shown a correlation between aluminum concentration in the human body and neurodegenerative diseases, such as encephalopathy, microcytic anemia and Alzheimer's disease (AD). Neurofibrillary tangles and senile plaques are the principal hallmarks of Alzheimer's disease. The presence of aluminum in neurofibrillary tangles is likely due to the interaction between aluminum and the hyperphosphorylated form of protein tau. Poised to investigate this interaction, synthetic studies were launched on the aqueous chemistry of aluminum with low molecular mass targets akin to AD biomolecules. These targets included organo-phosphonate and organo-phosphonate-carboxylate substrates. New crystalline materials emerged from the employed synthetic procedures and were characterized by elemental analysis, FT-IR and X-ray crystallography. Comparative studies in the solid state and in solution projected structure-reactivity relationships of the binary Al(III)-L species, thus reflecting their potential involvement in further ternary interactions relevant to neurodegenerative processes in Alzheimer's disease.

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FINAL SESSION

The D.J. Birchall memorial lecture

Chemical speciation, solubilities and surface complexation in Al(hydr)oxide systems

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A detailed understanding of the bioavailability, toxicity, transport and deposition of trace metals and inorganic/organic ligands in natural aquifers requires knowledge of their chemical speciation. Also the optimization of many industrial processes, e.g. within hydrometallurgy, mineral processing and pulp and paper processes relies heavily on the understanding of the chemical speciation in often complicated multicomponent, multiphase systems.
While the importance of solid/solution interfaces is well recognized, the ability to characterize their structure and properties and the processes they mediate is a great scientific challenge. An understanding of interfacial phenomena at the molecular level remains unclear for all but the simplest systems. At the heart of the problem is determination of the surface binding sites and chemical species involved in the interfacial processes. Therefore, ideally, the study of interfacial phenomena must involve measurements that give insight into which species that are present in solution and at the surface. Furthermore, possible dissolution/precipitation processes of the bulk solid phase and surface precipitates must be taken into account.
Recent applications of solution chemistry concepts to describe interactions at the particle - water interface have been very successful. The development of different surface complexation models has implied a better and more detailed understanding of sorption processes at work at hydrous particle surfaces.
Surface complexation has successfully been studied by means of high precision potentiometric titrations combined with appropriate sorption experiments. However, these experimental procedures yield just stoichiometries of the reacting components to form a surface complex and no information on a molecular level. Direct molecular level spectroscopic techniques (e.g. FT-IR, EXAFS, XPS, NMR) are therefore necessary to find out the coordination mode of the metal ion, the ligand and possible metal complexes.
The presentation will focus on the coordination chemistry of Al(hydr)oxide surfaces. Besides their acid/base and surface charge properties, composition, stability and surface coordination features with respect to complexation of organic ligands will be discussed.

ADDITIONAL POSTERS

SESSION 1. ALUMINIUM CHEMISTRY

Interaction of polyacrylic acid with dissolved Aluminium and boehmite (γ-AlOOH)

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We present experimental data on the interaction of polyacrylic acid (PAA) with dissolved aluminium and boehmite particles in NaCl media. The solution study involves high precision potentiometric titrations of PAA in the presence and absence of dissolved aluminium. Complexation is evident from the shift of the titration curves with increasing concentrations of aluminium. Beyond a given Aluminium to carboxylate ratio, precipitation of a gel-like phase was observed. Studies with the boehmite particles involved titrations of the particles in the absence and presence of PAA. The isoelectric point of the boehmite particles was obtained in electrokinetic experiments. Furthermore, adsorption experiments with PAA onto boehmite were carried out as a function of pH and total constant PAA concentrations or at constant pH and varying PAA concentrations. In some experiments dissolved Aluminium was measured. Adsorption was found to be strong over the whole pH range studied. The titrations show enhanced proton release in the presence of PAA compared to the pure boehmite – proton system all in agreement with the known pattern of anion adsorption to oxide minerals.

Fluorimetric study of the interaction between oxidized coenzyme II and Al(III)- ciprofloxacin complex in aqueous solutions

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In the tris-HCl buffer solution at pH 7.0, aluminum can enhance the fluorescence intensity of ciprofloxacin (CPFX) by forming a stable complex of Al(III)-CPFX. With the addition of oxidized coenzyme II (nicotinamide adenine dinucleotide phosphate, NADP) into the Al-CPFX solution system, the fluorescence intensity of Al-CPFX decreased remarkably. It is most likely that NADP was able to weaken the interaction between Al(III) and CPFX. Based on this, we initially assumed that there exist electrostatic interactions between Al(III) and NADP. It was confirmed by nuclear magnetic resonance spectroscopic experiments (^{1}H and ^{31}P NMR). This fluorescence quenching phenomenon was developed as an analytical method for the determination of trace amounts of Al(III) or NADP at physiological conditions.

Aluminum adsorption on titanium phosphate and silica-modified titanium phosphates

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Synthesis, characterization and sorption affinities of titanium phosphate (TiP) and titanium phosphate silicates (TiPSi) in relation to various metal ions were recently reported by our group. In this work, we explored further adsorption properties of these materials with respect to aluminium ions in aqueous solutions. Sorption of Al^{3+}(aq) increases with pH and reaches ca. 13 mg/g at pH close to neutral conditions and it can be described by an ion-exchange mechanism. Surface speciation of aluminium was studied by ^{27}Al and ^{31}P solid state NMR. These and other spectroscopic data were correlated with adsorption macroscopic measurements. Purification of process and drinking waters is one of the potential applications of these cheap adsorbents manufactured by decomposition of mineral sphene, $CaTiOSiO_4$, which is abundant up to 5 % in tailings of apatite ores in Kola peninsula.

SESSION 2. ALUMINIUM GEOCHEMISTRY

Laboratory assay of compounds transport through intact soil sample in controlled conditions

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Considerable attempt has been focused to aluminium mobilization in forest soils in relation to soil acidification. The rate and magnitude of leaching of Al and other elements and compounds from soils can be examined by means of percolation experiments.
Elements elutriation was studied in laboratory conditions by a percolation experiment using forest soil samples from Paličník area in the Jizera Mountains (Czech Republic) with different characteristics (soil type, soil horizon, and type of vegetation). Solutions with sulphates, nitrates and chlorides addition were used for percolation to simulate rainfall activity (under canopy and on open area). Passing liquid phase was analysed with respect to aluminium speciation, Mn and alkaline cations concentrations, and inorganic and organic anions concentration.
The acquired information was evaluated mathematically by the help of HYDRUS-1D simulation model. Differences between horizons and type of vegetation were found. The differences reflected various origin of organic matter content.

Soil aluminium pools assessment along three mountainous elevation gradients

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Anthropogenic soil acidification in mountain forests and consequent Al release present still a significant problem in many regions. The effect of deposition may differ according to stand conditions, including altitude. This contribution is focused on three elevation transects, two in the Jizera Mountains strongly influenced by acid deposition, one in the less affected Novohradske Mountains. Quantification of pools of different Al forms and related soil characteristics (organic carbon, exchangeable hydrogen cations, etc.) is evaluated. In the Novohradske Mountains, the pool of both organically bound and exchangeable Al increases with increasing altitudes. In the Jizera Mountains, the distribution is more complicated; it is strongly affected by different forest type (beech vs. spruce). Higher total amounts of Al are bound in the mineral horizons compared to surface organic horizons, even in the case of organically bound Al pools. Further differences between different altitudes were revealed by detailed Al speciation using HPLC/IC.

Comparison of meadows, forested meadows and forests soil characteristics in the Giant Mountains

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The Giant Mts. is a region impacted by human activities in the past. The cultivation of allochthonous ecotype of spruce in the form of clear-cutting system and high concentrations of acidificants in the atmosphere have led to forest and soil damage. The aim of this paper is to describe the influence of vegetation cover on the distribution of Al forms. The test sites include meadow, forested meadow and spruce forest soils. Basic soil characteristics, content and speciation of different Al forms (Al_{H2O}, Al_{KCl}) were determined. The results showed that the soils under grass cover have higher pH values and lower content of both Al forms compared to the adjacent forest soils. In all studied stands non-toxic Al forms were the most abundant forms released by water. The actual threat of Al is not serious but the disturbance of existing equilibrium can cause release of highly toxic Al^{3+} form to the soil solution.

Forms of Al in a soil in NW Spain amended with different sized limestone

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In order to compare the effectiveness of different sized lime, magnesium limestone of various particle sizes was applied to an acid soil in Galicia (NW Spain), destined for pasture production. The experimental design consisted of six treatments in total. They were: 2-4 mm, 0.5-2 mm, 0.25-0.5 mm and <0.25 mm limestone applied at a rate of 3 t ha^{-1} at the beginning of the experiment and <0.25 mm limestone applied in three 1 t ha^{-1} yearly applications plus the control. Each of them was replicated four times. The soil was monitored over a period of 3 years after liming and the forms of Al in the soil solid and liquid phases were determined. The experimental plots treated with a single application of the finest limestone (<0.25 mm) presented the highest pH and the lowest concentrations of exchangeable Al, with Al saturation lower than 10% throughout the period of study. These plots presented also the highest concentrations of organo-aluminium complexes in both the solid and liquid phases and the lowest concentrations of soluble monomeric aluminium, including Al^{3+} (considered the most toxic Al species). The presence of non-crystalline inorganic Al increased over time at the expense of exchangeable and/or organically-bound Al. The application of the finest limestone split in three sub-doses was less effective than the application of a single dose. The plots treated with the coarsest limestone (between 2 and 4 mm) and the control plots provided similar results throughout the three years of the study. In the former, the exchangeable Al was higher than 20% and the concentration of soluble inorganic monomeric Al was the highest of all plots.

Chemistry of soluble aluminium in forest and pasture soils upon fluoride addition

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It is known that atmospheric emissions of fluoride from aluminium smelters increase the content of fluorine in soils and vegetation in adjacent areas. Therefore, the effects of fluoride addition on the chemistry of soluble aluminium in soil samples from the area surrounding an aluminium smelter-alumina refinery located on the northern coast of Galicia (NW Spain) were investigated in laboratory experiments. Soil samples were brought to equilibrium with up to 5.26 mmol F L^{-1}. Addition of fluoride resulted in increased aluminium in the equilibrium solutions, particularly in forest soils. Interestingly, the increase of aluminium concentration goes along with an increase of solution pH, which results from the displacement of OH^- by F^-ions. Most aluminium in solution was bound to organic matter. In pasture soils, moderately acid, soluble aluminium consisted of non-reactive (acid-soluble) aluminium and organic monomeric Al. In forest soils, strongly acid, the non-reactive fraction is negligible. Inorganic aluminium complexes were found only in forest soils; along with Al-OH complexes, Al-F complexes constitute a considerable fraction of inorganic monomeric aluminium, particularly following high fluoride additions. On their turn, the aluminium-bound fluoride accounted for up to 80% of the total fluoride in solution in forest soils, while free fluoride predominated in pasture soils. AlF^{2+} is the only fluoride-aluminium complex at low fluoride additions. Upon higher F additions, AlF_2^+, AlF_3 and AlF_4^-, which are considered less toxic than Al^{3+} and Al-OH species, increased. Thus the rhyzotoxic Al-OH complexes are virtually absent at fluoride additions of 0.5 mmol F L^{-1} or more. The Al^{3+} ion, considered the most toxic aluminium species, became less prevalent as fluoride was added to the soil, reaching concentrations as low as 10^{-12} mmol L^{-1} at pH 5 or less. AlF_3 is the dominant among inorganic monomeric Al species at 5.26 mmol F L^{-1}. These results suggest that fluoride diminishes the toxicity of aluminium to plants (in spite of increasing total soluble aluminium), since the formation of fluoride-aluminium complexes reduces the availability of the most toxic species.

Spatial relationship and pools of acidificants in forest soils with different plant cover

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Increasing toxicity and mobility of aluminium is one of the dangers the forest ecosystems have to deal with. Aluminium, mainly in Al^{3+} form, in higher concentration affects the finest roots and thus negatively influences uptake of nutrients, water, and causes total weakening of plants themselves.
The set of soil samples taken in the long term field trial at area of Paličník Mountain in the Jizera Mountains enables to compare pools as well as spatial relationship of elements of interest between different forests stands (spruce and beech stands with clear-cut area). We measured concentrations and speciations of water soluble aluminium, amount of main anions (SO_4^{2-}, NO_3^-, Cl^-), content of organic carbon (Cox), its quality as A_{400}/A_{600}, and soil active and exchangeable pH. All the data were georeferenced and analysed by ArcGIS. The results presented in maps depicting spatial distribution of individual observed parameters yield to final comparison of different studied areas.

Aluminium pools and fluxes in forested catchments of two acidified mountain lakes

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We estimated major Al pools (soil, trees, and understory vegetation) and fluxes (precipitation, litter fall, and terrestrial export by surface water) in catchments of two acidified Bohemian Forest (BF) lakes, Plešné (PL) and Čertovo (CT). Depending on soil type, the BF soils stored 7–81 mol m^{-2} total Al, of which 10-50% was Al oxohydroxide, 5-10% KCl exchangeable Al, and ~0.1% water-extractable Al. Al pools in the BF forest and understory vegetation were 20–50 and 40–50 mmol m^{-2}, respectively. Average Al inputs on the forest floor were 0.5, 3.2, and 7.8 mmol m^{-2} yr^{-1} by throughfall deposition, decay of aboveground understory vegetation, and litter fall, respectively. The terrestrial Al export was 29–35 mmol m^{-2} yr^{-1}. Besides Al leaching from mineral soil horizon, the litter horizon (with pools of water-extractable Al between 0,1–0,15 mmol m^{-2} and permanent Al input by deposition and litter fall) may be an important Al source, especially during lateral flow events.

Aluminum biogeochemistry of a forest catchment underlain by leucogranite in western Bohemia

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The 27-ha Lysina catchment forested by Norway spruce (Picea abies) is very sensitive to acidic deposition due to slow weathering of the base-poor rock. Water fluxes were monitored continuously between 1990 and 2008. Inputs of strong acids were not completely neutralized in the soil and the soil water was strongly acidic. As a result, ions of H^+ and Al^{n+} were important cations in drainage water especially in high-flow periods. Potentially toxic inorganic monomeric Al (Alim) was the dominant fraction of stream water. Species of Alim were mainly alumino-fluoride complexes, aquo Al^{3+}, and alumino-sulfate complexes according to the MINEQL+ modeling. Long-term declines of total Al (Altot) and Alim attributed to the acidification recovery were observed in stream water. Low biodiversity of benthic macroinvertebrates was documented in the stream sediments. Observed needle yellowing may be linked with low Mg content of needles and high Al content in soil solutions and fine roots.

Aluminum biogechemistry of a forest catchment underlain by serpentine in western Bohemia

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The 22-ha Pluhuv Bor catchment forested mainly by Norway spruce (Picea abies) is very resistant to acidic atmospheric deposition due to intense chemical weathering of the ultramafic magnesium silicate rock. The site was studied continuously between 1992 and 2008. Mineral weathering generated near neutral magnesium-bicarbonate-sulfate stream water. Mean stream water concentrations of total Al (Altot) were very low in the first half of the 1990s reflecting high pH. Concentrations of Altot increased markedly in the 2000s. However measured Al speciation did not show any significant increase in inorganic monomeric Al (Alim) and organic monomeric Al (Alom) and both monomeric fractions exhibited very low concentrations. Therefore, most of Altot increase in stream water and soil water was made up from colloidal and particular Al fractions. The fraction of Alim was entirely dominated by complexes of Al-OH according to the MINEQL+ modeling.

Aluminium mobility in representative forest soil profiles in the Jizera Mountains

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Soil acidification (due to acid deposition, acid parent rocks, high precipitation, and specific vegetation) presents a serious problem mainly in forest soils. It can result in a release of potentially toxic Al forms. To predict potential Al leakage into the groundwater, soil water regime and substances transport may be simulated using the numerical models. Typical acid soil profiles (Podzols, Cambisols) present in the Jizera Mountains under various plant cover (spruce forest, beech forest, grass) were chosen to asses potential groundwater contamination in this area. Soil hydraulic properties and properties affecting dissolved substances transport of all diagnostic horizons were studied in the laboratory. The numerical model HYDRUS-1D was applied to simulate water and Al transport within the soil profiles. Soil water regime and Al transport reflected various retention ability and different sorption properties of studied soils, which were significantly affected by organic matter origin (plant cover).

Trends of aluminium concentration and acidity of soil solution at highly acidified spruce stand in Ore Mts.

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Deposition and soil solution chemistry have been measured at Nacetin stand (spruce forest) since 1994. Throughfall deposition of sulfur (S) declined from 48 kg ha^{-1} yr^{-1} in 1994 to 16 kg ha^{-1} yr^{-1} in 2007. Bulk deposition of inorganic nitrogen (N) was about 11 kg ha^{-1} yr^{-1} during the observed period. As a result of anthropogenic acidification of soil, low pH and high concentration of aluminium was measured in soil solution (tension lysimeters). Despite high reduction of incoming acidity, soilwater pH did not change significantly. Soil solution pH in 30cm was about 4.3, pH = 4.2 in 60 cm and 4.35 in 90 cm between 1995 and 2007.
On the other hand aluminium concentration decreased significantly from 310 μmol L^{-1} in 1997 to 130 μmol L^{-1} in 2007 at 30 cm, from 230 μmol L^{-1} in 1997 to 140 μmol L^{-1} in 2007 at 60 cm and from 250 μmol L^{-1} in 1995 to 120 μmol L^{-1} in 2007 at 90 cm. Decrease of aluminium concentrations was caused by reduction of inorganic anions (SO_4^{2-} + NO_3^-). Concentration of sulfate and nitrate explained 95, 90 and 80% of variability in aluminium concentration at 30, 60 and 90cm depth, respectively. Observed decrease of Al concentration did not follow gibbsite or other Al-bearing minerals solubility control, Al decrease was a result of ionic strength reduction of soil solution.

Changes of Al and Fe forms in the scope of pedogenic processes in naturally developing fir–beech forest

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The study is focused on changes in Al and Fe behaviour with respect to time and in the scope of pedogenic processes in naturally developing fir–beech forest Razula (Western Carpathians). Soil samples from 8 different windthrow sites with known times of catastrophic event were tested; samples were taken from six depths from three parts of the windthrow - pit, mound and undisturbed part as a control. Exchangeable ("free"), oxalate extractable, dithionate-citrate-bicarbonate extractable forms of Al and Fe were measured. Relationships with other chemical analyses were studied. The results obtained were confirmed by the X-ray diffraction. It was found that contents of Al and Fe forms differed with respect to time and soil sample location. Forms of Al and Fe determined for the oldest windthrows are similar to those measured in undisturbed parts. On the contrary, the Al and Fe forms in recent windthrows showed significant differences in disturbed and undisturbed parts of the windthrow.

SESSION 3. ALUMINIUM AND PLANTS

Relationship between aluminum and major metals in root zone soil solutions of buckwheat (*Fagopyrum esculentum* Moench), an Al-accumulator

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The fluctuation and relationship of aluminum and other major metals including Ca, Mg, Mn and K in root zone soil solutions were studied by growing buckwheat (*Fagopyrum esculentum* Moench. cv. Jiangxi), an Al-accumulator, in acid soils amended with $CaCO_3$ at the concentration of 1.0 g per kg soil and non-amended controls. Soil solutions were collected with a preinstalled nondestructive soil solution sampler every 7 d. The results showed that pH value of the soil solutions was not the only factor controlling the concentration of Al, while Ca and Mg played important roles during the process of Al uptake by buckwheat from soil solutions. However, the ratio of base cations to aluminum (BC/Al, (Ca+Mg)/Al) alone is not enough to interpret the high Al-tolerance of buckwheat. The fluctuation of metal elements including Al, Ca, Mg and Mn is a holistic effect of the competition among Ca-Mg-H-Al, the chelation of oxalate and other organic acids secreted from roots, and the uptake of buckwheat.

The effects of aluminium toxicity and low pH on early ontogenesis of *Isoëtes echinospora*

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A population of *Isoetes echinospora* survived a thirty-year period of severe acidification in Plešné Lake (Bohemian Forest, Czech Republic) under high concentrations of phytotoxic aluminium (Al). Sporeling survival and age structure were examined during population recovery in 2004–2008. Laboratory experiments were conducted to assess the effect of various pH (4–8) and Al concentration (0–1000 μg l^{-1}) on sporeling ontogenesis. The experiments clearly showed that Al concentration ≥300 μg l^{-1}, as well as high acidity (pH=4), considerably reduced absorptive organs (macrogametophyte rhizoids, roots and root hairs) and inhibited sporeling growth. The ratio of the belowground to the aboveground biomass dropped below 1, which caused serious impairment of the quillwort life strategy of root system dominancy. The specific responses of the lake sporelings corresponded with those of the experimental sporelings exposed to 100–300 μg l^{-1} of Al laboratory treatments and reflect the Al toxicity of the lake water. As the establishment and survival of quillwort sporelings had been adversely affected over the long acidification period, a greater individual longevity of adult plants was responsible for the population endurance in Plešné Lake.

SESSION 4. ALUMINIUM AND THE ENVIRONMENT

Determination of trace amounts of total aluminium and aluminium species in environmental samples by spectrometric methods

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During the last few decades aluminium and its distribution in the environment has attracted much attention. Its concentration in different environmental samples varied widely. While determination of high aluminium concentrations can be realized with no serious problem, reliable determination of its trace and ultratrace concentrations requires the development of enrichment techniques capable of improving both, the selectivity and also sensitivity for this analyte. In this work, two extraction techniques were used for selective separation and preconcentration of trace aluminium concentrations. Solid phase extraction (SPE) using nanometer-sized titanium dioxide as a solid sorbent and cloud point extraction (CPE) using nonionic surfactant octylphenoxypolyethoxyethanol (Triton X-114). Spectrometric methods such flame (FAAS) and electrothermal (ETAAS) atomic absorption spectrometry, inductively coupled plasma combined with optical emission spectrometry (ICP-OES) or mass spectrometry (ICP-MS) were applied for the determination of aluminium after its selective separation and preconcentration. Optimized procedures were used for the determination of trace and ultratrace amounts of total aluminium and aluminium species in speciation and fractionation studies using environmental samples such as natural waters, soils and sediments.

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Study of aluminium biosorption and bioaccumulation by fungal biomass

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Biosorption is a property of certain types of inactive or dead microbial biomass to bind and concentrate the metals from dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger or extracting solid phase of biological origin. It is particularly the cell wall structure of certain algae, fungi or bacteria which was found responsible for this phenomenon. Opposite to biosorption is metabolically driven active bioaccumulation by living cells. That is an altogether different phenomenon requiring a different approach for its exploration. Anyway the biosorption and bioaccumulation can be defined as a physico-chemical interaction which may occur between metal and cellular compounds.
Biosorption and bioaccumulation of aluminium from synthetic solutions, natural waters and soil extracts was studied using the biomass of microscopic filamentous fungi. The recoveries of studied processes were quantified by the spectrometry methods. The changes of Al(III) speciation and pH values, the fungi resistance to Al and the influence of fungi metabolism on the studied processes were investigated also.
In certain conditions the studied fungi can be used as the bioanalytical tools for *in-situ* separation and preconcentration of bioavailable species of aluminium or their operationally and/or functionally defined fractions directly in the ecosystem, e.g. in the analysis of polluted waters. The biotechnological application of the remediation techniques by long-term metal bioaccumulation by fungal biomass presented in contamined areas affected e.g. by acidification processes from mine activity can markedly help in the ecosystem revitalization.

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Use of sodium silicate to reduce aluminium toxicity towards fish

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In acidified water cationic aluminium-species (Ali) are toxic towards fish, due to deposition and clogging on gills causing ionregulation and/or respiratory problems. We wanted to study if sodium silica could counteract Al toxicity both in Al-rich freshwaters and in estuaries receiving acid and limed water. Utilizing i*n situ* Al fractionation techniques and exposure of fish in tanks, the change in Al toxicity following the addition of sodium silicate was studied. By adding sodium silicate to acid Al rich water, the retention of Al-species (Ali) in Chelex 100 resin as well as the Al accumulation on fish gills decreased. Upon mixing with seawater, the Ali concentration in water and the concentration of accumulated Al on gills were lower than in corresponding estuarine mixtures without excess silicates. However, addition of sodium silicate to limed Al-rich river water increasing pH >7, resulted in increased accumulation of Al on gills. Thus, the use of sodium silicate to counteract Al toxicity towards fish might be limited to acid freshwaters.

Comparison of aluminium in alkaline and acid water due to accumulation on fish gills

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The toxicity of $Al(OH)_4^-$ (aluminate) towards fish, which predominates in alkaline waters, is poorly understood. In acid water it is well established that the predominant positively charged Al-species are toxic towards fish due to accumulation on fish gills, causing ion regulation and/or respiratory problems. By exposing fish to aluminate in alkaline water (pH 8.8) and parallel, exposing fish to the same Al concentration in acid water (pH 5.4), the gill reactivity and effects of aluminate were compared to gill deposition and effects of Al-cations. Based on *in situ* fractionation of Al-species in waters and sampling of gills from exposed fish, results showed that aluminate was present as LMM Al species that were retained in the Chelex 100 exchange resin and accumulated on gills, similar to that observed for Al-cations from acid water at equal concentrations. Measured stress responses in fish indicate no difference between the different Al exposures.

SESSION 6. ANIMAL MODELS OF ALUMINIUM TOXICITY

Aluninum combined chelation with a glycosyl-mono- and a bis-hydroxypyridinone. Solution and biodistribution Studies

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To minimize acute toxic effects associated with aluminium accumulation in human body, namely in patients with dialysis encephalopathy and some forms of osteomalacia, chelating therapy has been used. The drawbacks associated with the most current Al-chelating drug (Deferrioxamine, DFO) and the disclosure of Deferriprone (DFP) as drug for iron-overload chelation therapy, make the 3-hydroxy-4-pyridinone (3,4-HP) based compounds important pharmaceutical targets. The use of combination therapy with two chelators, namely DFO and DFP, has also been recently explored for Iron and Aluminum decorporation.
Taking into account this recent poly-pharmacological strategy, we have explored a combined chelation based on 3,4-HP compounds with complementary differences in terms of denticity and some physico-chemical properties. We describe herein the results of a combined Al-chelation using a tetradentate bis-(3,4-HP) amino acid and a glycosyl 3,4-HP derivative. Therefore, studies on aluminum complexation and speciation were undertaken in solution as well as in vivo assessment on the ability for the removal of a radiotracer (^{67}Ga) from loaded mice, as a model of Aluminum accumulation in body. Animal studies have shown that the simultaneous administration to metal-loaded mice of this pair of HP chelators, under appropriate ratio, can speed up the metal excretion, as compared with that of the individual chelators. This may be rationalized by coadjuvation and eventual synergistic effects, due to complementary accessibility of the chelators to different cellular compartments.

Role of deferroxamine in some enzymatic stress markers in AßPP transgenic mice exposed to aluminium

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Aluminium (Al) is a widely environmental toxicant and a neurotoxic agent implicated in neurodegenerative diseases. Al induces the production of free radicals. The accumulation of free radicals may account for degenerative events of ageing such as Alzheimer's disease. On the other hand, deferoxamine (DFO), an iron and aluminium chelating agent has been widely used to facilitate removal of iron and chronic aluminium overload. In order to evaluate levels of metal concentration and differences in genotype oxidative response after oral exposition of Al and DFO treatment, AßPP transgenic (Tg2576) and C57BL6/SJL (wild-type) mice of 5 months of age were fed with aluminium lactate supplemented in the diet (1 mg of Al/g diet) and simultaneously received subcutaneous 0,20mmol/kg/day twice a week until the end of the study at 11 months of age. Four groups of treatment were carried out: control; Al; DFO; Al+DFO respectively for Tg and wild-type animals. At the end of the treatment, hippocampus, cerebellum and cortex were removed and processed to examine the following oxidative stress markers: GSH, GSSG, SOD, GR, GPx, CAT, and TBARS. The biochemical changes observed indicate that Al could not act as pro-oxidant agent and that the main oxidative effects are observed in the presence of DFO predominantly in hippocampus and cortex.

SESSION 7. HUMAN EXPOSURE TO ALUMINIUM

Dietary aluminium in coffee as affected by coffee type and beverage preparation method

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Coffee contains relatively high amount of aluminium due to the environment where the coffee tree is grown. This element is considered to be toxic for human. The methods of preparing coffee are different, however tools used for preparation are very often made from aluminium alloys or pure aluminium with very different quality. In the experiment seven types of coffee (LAVAZZA Espresso, Trung nguyen house blend, Trung nguyen ground coffee, Tchibo gold selection, Douwe Egberts 100% arabica, Trung nguyen ching phuc, Marila ground standard) were prepared by 3 ways usually used in the world (coffee infusion, percolation, coffee maker). First results show that low but significant differences in the content of aluminium and selected heavy metals in final coffee infusion exist due to the way of preparation and coffee type.

Aluminium in the water of the neogene-paleogene aquifer of Wielkopolska, Poland

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The Neogene-Paleogene aquifer plays an important role in supplying the population of Wielkopolska, Poland, with water, both potable and for domestic purposes. This especially concerns the areas where there are no aquifers in the Quaternary layer, or where the level of water in the existing aquifers is low, or their chemical composition is of poor quality. The hydrogeochemical environment of the Neogene-Paleogene aquifer displays considerable variability determined by geogenic factors and manifested by large differences in water chemistry, especially its colour, hardness, and the levels of chlorides, iron and manganese. The differences in quality are particularly significant in the region of the Poznań-Oleśnica graben, where water ascends from the deeper Mesozoic aquifers. In order to tackle the problem, in the years 2006-2008, studies of aluminium in the water of 133 wells located in the concerned zone were conducted. The aluminium determinations were performed using ICP-MS. The conducted research demonstrated the variability of aluminium concentrations in ground water of the Neogene-Paleogene layer in the area of central Wielkopolska. Higher aluminium concentrations were determined in the water ascending from the Mesozoic bed in the Poznań-Oleśnica tectonic dislocation zone and in the area of the ascending inflow caused by intensified and direct exploitation.

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Aluminium in tea, coffee and other plant stimulants and their decoctions

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The tea plant (*Camellia sinensis*) is a natural aluminium accumulator and consumption of infusion prepared from its leaves is one of major source of Al in human diet and can more than double an individual's intake. However, during the last decades, the tea is often replaced by other plant stimulants available from the world market.
Therefore we decided to evaluate amounts of Al in dry materials and infusions of 35 samples of traditional plant stimulants (tea and coffee) non-traditional plant stimulants (mate, rooibos, honeybush, and chamomile) and certain tea supplements (hibiscus, rose hip, ginkgo and lemon grass). The aluminium content was determined by means of inductively coupled plasma optical emission spectrometry.
Our results confirmed aluminium being the most abundant in the tea infusions, exceeding 2 mg/L, followed by hibiscus with almost 1 mg/L, whereas the highest amounts in the raw materials 1860 and 1000 mg/kg were determined in coffee and chamomile respectively.

Evidence of aluminium in newly-formed bone of osteoporotic patients

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Bone remodelling is a coupled process of bone resorption by osteoclasts and new bone formation by osteoblasts. In renal disease, aluminium (Al) accumulation interferes with these activities, giving rise to vitamin D-resistant osteomalacia and aplastic bone disease. Non-renal patients on long-term total parenteral nutrition are also prone to bone disease involving Al accumulation. The possible involvement of Al in osteoporosis has been less researched and is, consequently, less well understood. In this study, we examined the effects of Al on bone formation in osteoporotic patients diagnosed by low bone densitometric measurements (n=17), and normal controls (n=16), with an average age of 48 years (range 21-76 years). The osteoporotic patients studied here were younger and less severely affected than most osteoporotic patients. Their bone tissue had a smaller percentage of Al-stained bone surface (7.5%) than seen previously (30%) in more severely affected cases. Bone formation rates were assessed by non-invasive measurements of serum osteocalcin and by quantitative static and dynamic histomorphometry in cancellous bone biopsy specimens obtained from the iliac crest. Analyses included the percentage of Al-stained bone surfaces (Walton stain, UV light excitation), total osteoid surfaces, osteoid thickness (toluidine blue), osteoclast surfaces (acid phosphatase), and bone formation rate or BFR (tetracycline fluorescence) which is the product of the percentage of double-labelled tetracycline surfaces (% DLS) and the mineral apposition rate (MAR). In this group of subjects, the mean BFR for the osteoporotic patients was 0.03μm^2/um/day and for the controls 0.06 μm^2/um/day, a difference that did not reach statistical significance. While the percentage of Al-stained surfaces correlated positively with BFRs in the osteoporotic patients (r = 0.79, p< 0.0005), this was not the case for controls (r = 0.277, p = 0.30). Al staining in osteoporotic bone specimens also correlated with the total osteoid surfaces (r = 0.63, p< 0.01), % DLS (r = 0.63, p< 0.001), MAR (r = 0.76, p< 0.0005), and serum osteocalcin (r = 0.933, p< 0.0000005), and osteoclast surfaces (r = 0.51, p< 0.05). Al staining and BFRs lacked correlation with their age, serum parathyroid hormone and 1,25 dihydroxy-vitamin D levels. A comparison of paired bone sections (one unstained for tetracycline fluorescence and the other stained for Al) demonstrated that Al predominated at the sites where new bone formation occurred. On Al-stained bone surfaces, osteoblasts and osteoclasts were Al-positive, some exhibiting cytopathological change. In this younger group of osteoporotic patients, a strong correlation was found between bone Al deposition, histomorphometric markers of bone formation and non-invasive serum osteocalcin levels. *In vitro*, bone cells exposed to Al behave in a biphasic dose-dependent manner. It is suggested that in osteoporosis, in the first phase of a biphasic response, Al stimulates and incorporates into newly-formed bone. In the second phase, continued Al uptake into osteoblasts and osteoclasts would interfere with their ability to function.

SESSION 8. ALUMINIUM, ALZHEIMER'S AND OTHER NEUROLOGICAL DISEASES

Aluminum interaction with human brain microvessel endothelial cells (hBMECs), neurocapillaries and the neural vasculature of transgenic (Tg2576) mice fed aluminum-supplemented diets

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Objective: The vasculature of the CNS consists of dense networks of blood vessel microcapillaries composed largely of human brain microvessel endothelial cells (hBMECs), tightly associated neurons and glia, and other neural support cells. Single hBMECs, both *in vitro* and *in vivo*, are capable on their own of forming 5 μM diameter vascular tubes. The tandem association of hBMECs is thought to form the structural basis for microcapillaries and the blood brain barrier. The high metabolic rate of brain cells and nervous tissues in general make hBMECs critical regulators of nutrient transport and delivery of oxygen to neuronal, glial and other support cells, however, they are also important conduits for neurotoxins supplied via the circulation. In this study we examined the effects of potentially neurotoxic metal sulfates including aluminum, copper, iron, lead and zinc, using magnesium as a control, on hBMEC inflammatory signaling and gene expression, reactive oxygen species (ROS) generation, cellular apoptosis and cellular viability. We also examined brain vasculature obtained from Alzheimer's disease (AD) and age-matched control patients, and neural blood vessels from amyloid over-expressing transgenic (Tg2576) mice receiving diets enriched in aluminum-sulfate.
Methods: short post-mortem human brain neurovasculature; hBMEC cultures; ROS determination using 2',7'-dichlorofluorescin diacetate assay; electrothermal atomic absorption (EAE); Tg2576 (amyloid over-expressing) mice; apoptosis staining using Hoescht 33258 pentahydrate (bis-benzimide) assay; DNA array; RT-PCR; Northern & Western assay; bioinformatics; statistical analysis.
Results: When compared to age-matched controls, specific anatomical regions of AD neurovasculature were found to be enriched in aluminum deposition. In these same blood vessels isolated from AD brain there were no significant increases in copper, iron, lead or zinc. In contrast to copper-, iron-, lead- magnesium- and zinc-sulfate, aluminum-sulfate induced robust inflammatory, apoptotic and morphological changes in hBMEC cultures. Inflammatory gene expression patterns were also observed in aluminum-stressed hBMEC cells but not in parallel experiments using hBMEC cells and equivalent nM concentrations of other sulfates. Aluminum fed Tg2576 mice exhibited regionalized neuropathology that correlated positively to increased aluminum signals in brain vasculature as assayed by EAE.
Conclusions: *In vitro* and *in vivo* AD modeling continues to support a major causal role for aluminum in the regional development of AD-type neuropathology and the propagation of the AD process.